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Microbiota analysis of dried fruits and stress modelling in *Escherichia coli*

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1. BACKGROUND OF THE WORK AND ITS AIMS

In recent years, dried fruits have gained importance in modern diets due to growing consumer interest in healthy food products. They are concentrated forms of fresh fruits, processed either by traditional methods, such as sun-drying, or by advanced mechanical dryers (Ringeisen et al., 2014). Although dietary guidelines emphasise fresh, seasonal products, several countries, including Lithuania and the United Kingdom, also recommend daily consumption of dried fruits (Web1). Due to climatic limitations, year-round availability of fresh fruits is restricted, thus, dried fruits offer a valuable alternative. They are rich in dietary fibres, vitamins, minerals, and bioactive compounds that support digestive and immune functions and lower cardiovascular risk (Jeszka-Skowron et al., 2017; Rybicka et al., 2021; Alasalvar et al., 2023; Zeng et al., 2023).

The drying process reduces moisture content and water activity (a_w), thus inhibiting microbial growth and extending the shelf life of the products (Średnicka-Tober et al., 2020). Traditional dried fruits contain no added sugar, while others, such as cranberries or mangoes are sweetened to enhance the flavour and further reduce the water activity (Alasalvar et al., 2020). Despite typically low a_w (around or below 0.60), dried fruits are not free from microbial contamination (Beuchat et al., 2011). Pathogens such as *Listeria monocytogenes*, *Escherichia coli*, *Staphylococcus aureus*, *Salmonella* spp. and others can survive under these conditions. These contaminants have been isolated from commercial and home-dried products as well (Risiquat, 2013; Ntuli et al., 2017; Canakapalli et al., 2021; Shah et al., 2022, Abed, 2025). As dried fruits are often consumed without further processing, microbial and mycotoxin contamination remain major safety concerns (Zakaria et al., 2015).

Microbial detection traditionally relies on culture-based methods, including several selective media and biochemical tests, and advanced identification tools, such as MALDI-TOF MS. However, culture-based approaches are labour-intensive and may underestimate unculturable microbial diversity, as not all organisms can grow under standard laboratory conditions (Biswas and Rolain, 2013). Culture-independent methods such as the 16S rDNA amplicon sequencing, or quantitative PCR (qPCR) enable the detection of both culturable and unculturable microorganisms directly from food, environmental or clinical samples). However, biases in DNA extraction, the choice of primers, target genes and reference databases significantly can affect the accuracy of taxonomic identification (O'Sullivan et al., 2011; Schoonbroodt et al., 2023). Combining both approaches provides the most comprehensive characterisation.

Environmental stressors, including heat, low temperature, drying, high osmolarity, chemical preservatives, irradiation, or combinations such as freeze-drying can induce bacterial dormancy ((Fu et al., 2020; Li et al., 2020; Cai et al., 2022; Jayeola et al., 2022; König et al., 2023; Pazos-Rojas et al., 2023; Hu et al., 2024; Shangguan et al., 2025). Besides spores and persistent cells, the viable but non-culturable (VBNC) state is especially relevant, as VBNC cells remain metabolically

active yet using standard culture methods these cells remain undetected. Thus, their detection requires molecular approaches such as qPCR or 16S rRNA sequencing (Li et al., 2014).

In high-osmolarity environments (hyperosmotic stress), bacteria must adapt quickly to maintain turgor pressure and intracellular hydration. To counter this, cells accumulate compatible solutes (osmoprotectants), such as glycine betaine, carnitine, proline betaine, and trehalose (Breisch and Averhoff, 2020). In *E. coli*, osmotic stress triggers a complex response involving gene regulation, metabolic shifts, and solute accumulation (Sévin and Sauer, 2014; Sun et al., 2019).

Gamma irradiation is an emerging non-thermal preservation method that uses high-energy photons (typically from Cobalt-60 or Cesium-137) to inactivate microorganisms (Singh et al., 2015). At sufficient doses, irradiation damages microbial DNA. Importantly, at lower doses (≥ 4 kGy) gamma radiation has only minimal effect on food macronutrients, micronutrients, flavour, and texture (Hing et al., 2022; Rafiepour et al., 2024). Higher doses can be responsible for the degradation of lipids, causing undesirable odours and rancid flavours (Huang et al., 2023). It can be applied to packaged products, thereby preventing post-treatment contamination (Pelicia et al., 2015). It also delays ripening, inhibits sprouting, and controls insects (Yoon et al., 2023). Although recognised as safe by WHO and FAO, the acceptance varies by country. Given that osmotic stress in dried fruits may promote the VBNC state, understanding how gamma irradiation affects such pre-stressed microbes is essential for ensuring food safety (Web2).

While fresh fruits have been extensively studied for their microbial communities, less is known about the microbiota of dried fruits. Several studies have demonstrated that unhygienic conditions at any point in the food chain can lead to contamination with pathogenic bacteria, including *Escherichia coli*, *Enterobacter*, *Salmonella*, *Listeria*, and *Staphylococcus*, raising food safety concerns. *Escherichia coli* was selected for this thesis as an indicator of faecal contamination in dried fruits, and as a model organism for studying osmotic stress. To investigate the composition of the microbiota and evaluate how sugar induced osmotic stress influences *Escherichia coli*, I conducted a series of experiments with the following objectives:

- Characterize the microbial composition of commercially available dried fruits (dried apricot, prune, and raisin) from Hungary and Austria, with special emphasis on detecting members of the Enterobacteriaceae family, and more specifically, *Escerichia coli*,
- Perform growth studies using the Bioscreen system to determine whether *E. coli* is capable of proliferating under sugar-rich conditions,
- Investigate whether the osmotic environment found in dried fruits induce a viable but non-culturable-like state in *E. coli*,
- Examine a broader view of *E. coli*'s adaptation to sugar-based osmotic stress in dried fruit-mimicking environment. Three target genes were selected to represent different aspects of the osmotic stress response: the *osmC*, *talA*, and *treA*.
- Assess the effect of ionizing radiation on *E. coli* under osmotic stress conditions.

2. MATERIALS AND METHODS

2.1 Collection of dried fruit samples

Thirty dried fruit samples (apricots, prunes, raisins) were purchased from Hungarian and Austrian markets and covered a broad range of product types (packaged/unpackaged, organic/non-organic, SO_2-treated/untreated). Samples were stored at room temperature. The work was carried out within a MATE–BOKU collaboration (CEEPUS) and a Hungarian Austrian bilateral project, which is why samples came from both countries.

2.2 Selection of *Escherichia coli* strains

Two *E. coli* strains were used, a culture collection strain (CC; ATCC B.02031), and a food isolate (FI) previously obtained from a low water activity food (buckwheat flour). The FI strain was incidentally isolated in an independent study during *Cronobacter* sp. screening of low water activity food products. After enrichment and plating on selective media, MALDI-TOF MS identified *E. coli*. The isolate was confirmed through re-culturing and repeated MALDI-TOF analysis.

2.3 Growth potential determination

Growth of CC and FI strains was measured using the Bioscreen C system. Strains were tested in Luria-Bertani medium (LB) (control), a low-sugar medium (0.35 M glucose), and media mimicking the sugar composition of dried apricot, prune, and raisin. Experiments included 24 h, 72 h, and 96 h incubations in these media. In one of the experiments, osmotically pre-adapted *E. coli* cells in prune-mimicking medium were used. Statistical analysis was performed with non-parametric tests (Kruskal–Wallis and Wilcoxon).

2.4 Culture-dependent microbiota analysis

In this experiment, two approaches were used. The first was the qualitative detection of aerobic mesophilic microbes, with emphasis on detecting the members of the Enterobacteriaceae family, more specifically, *E. coli*, using homogenization, an enrichment step (in MMGM and TSB), and plating on selective (TBX, VRBG) and non-selective (LB) agar. The second approach was a quantitative enumeration without the enrichment step, using serial dilutions and plating on agar plates supplemented with Actidion. This was added to the medium to inhibit yeast and mould growth, thereby enabling the accurate determination of the total mesophilic bacterial cell count.

Bacterial and fungal isolates from the plates were identified using MALDI-TOF MS. Representative colonies were selected from each plate, prepared following standard formic-acid extraction procedures, and identified using Bruker spectral libraries.

2.5 Additional tests for microbiota characterization

To better understand the microbiota of dried fruits, the pH, water activity, and the sugar content of these fruits were analysed.

2.6 16S rDNA amplicon sequencing (culture-independent analysis)

To complement culture-based identification, bacterial community composition was also determined using the 16S rDNA amplicon sequencing. For this, the first step was the DNA extraction, where Genomic DNA was extracted using the DNeasy PowerFood Microbial Kit via chemical lysis, bead-beating, and purification. The 16S gene (~1,500 bp) was amplified using the Oxford Nanopore 16S Barcoding Kit and AccuStart II PCR SuperMix, following the manufacturer's protocol. The amplicon size was confirmed on a 2 % agarose gel stained with GelRed. The quantity of the DNA was validated with Qubit fluorometer. Barcoded libraries were prepared using the rapid sequencing DNA - 16S barcoding kit 24 V14, normalized, pooled, purified with AMPure XP beads, and loaded onto the flow cell of the MinION sequencer.

2.7 Stress induction by ionising radiation

2.7.1 Comparison of e-beam and gamma irradiation

A preliminary experiment was conducted to compare the effects of electron-beam (LINAC) and gamma irradiation on the CC strain of *E. coli*. The aim was to determine its D-value. An overnight culture of the CC strain of *E. coli* was prepared in LB medium and incubated at 37 °C. On the day of the experiment, the optical density at 600 nm (OD_{600} value) of the cultures was adjusted to 0.1 using fresh LB medium. The samples were irradiated at doses of 0.2, 0.4, 0.6, 0.8, and 1 kGy at the Wigner Research Centre for Physics. After treatment, samples were serially diluted, plated on LB agar, and incubated for 24 h at 37 °C.

2.7.2 Determining the D-value of CC strain of E. coli with e-beam irradiation

Based on results from the initial comparison, the D-value for e-beam irradiation was determined using doses of 0.5, 1, 1.5, and 2 kGy. The sample preparation, the plating, and the incubation followed the protocol described above.

2.7.3 Assessing the behaviour of E. coli in osmotic environments resembling dried fruit conditions upon gamma irradiation

This experiment assessed the survival of *E. coli* in media containing glucose, fructose, or sucrose at concentrations of 0%, 10%, 30%, and 50%. Cultures were adjusted to OD_{600} = 0.1 in the media with different sugar composition and concentration, and were irradiated with gamma rays, at doses of 1, 1.5, 1.8, 2, and 2.5 kGy. After treatment, samples were serially diluted, plated on LB agar, and incubated for 24 h at 37 °C.

2.7.4 Determination of the D-values of CC and FI strains of E. coli using gamma irradiation

Radiation doses were adjusted to 1.3, 1.6, 1.9, 2.2, and 2.5 kGy, and both the CC and the FI strains were irradiated in LB and prune-mimicking medium. All preparation, plating, and incubation steps followed previous experiments.

2.8 Primer design for quantitative PCR (qPCR) studies

Primers for *rpoB*, *osmC*, *treA*, and *talA* were designed using Primer3web. Secondary structure formation was evaluated using OligoAnalyzer, and specificity was checked by NCBI BLAST. Gradient PCR (55–61 °C) was used to determine the optimal annealing temperatures of the primers. Reactions were run in 25 µL volumes with AccuStart II PCR SuperMix and analysed by agarose gel electrophoresis.

2.9 Detection of viable but non-culturable (VBNC)-like cells

To determine whether *E. coli* cells enter a viable but non-culturable (VBNC)-like state under different periods of osmotic stress, first a protocol was established. The osmotic stress experiments consisted of two parts.

2.9.1 The induction of osmotic stress without pre-adaptation period (viability and culturability assay)

To determine whether osmotic stress induces a VBNC-like state, the OD_{600} values of the overnight cultures (CC and FI strains of *E. coli*) were set to 0.1 in LB medium, and in media containing varying types and concentrations of sugars that resemble the sugar concentrations of dried fruits (dried apricot, prune, raisin). The samples were incubated for 1, 2, 3, 4, 5, 24, and 96 hours. This incubation represented the stress period. LB medium served as control. After each time point, a part of the samples was plated for the assessment of the culturability, and the rest was frozen for later DNA extraction.

2.9.2 The induction of osmotic stress with pre-adaptation period

To examine the effect of pre-adaptation, CC cultures were first pre-adapted for 2.5 h in LB and in low-sugar medium (0.35 M glucose). Control samples were plated after pre-adaptation. Cells were then exposed to dried fruit-mimicking media for 4 and 24 h. After each time point, samples were plated for culturability assessment, and aliquots were frozen for later DNA extraction.

2.9.3 DNA extraction from stressed cells, and propidium monoazide quantitative PCR (PMA-qPCR)

DNA extraction followed the peqGOLD Bacterial DNA Kit, with a propidium monoazide (PMA) step to eliminate DNA from dead cells. After PMA treatment and photoactivation, samples underwent lysozyme, proteinase K, and RNase treatment.

The SYBR Green-based qPCR was performed in 25 µL reactions with template DNA, *rpoB* (housekeeping gene) primers, and no-template controls. The cycling conditions included an initial denaturation at 95 °C for 60 seconds, followed by 40 cycles of denaturation at 95 °C for 15 seconds, annealing at 57 °C for 30 seconds, and extension at 72 °C for 30 seconds. Fluorescence detection was performed at the end of each extension step. Melt-curve analysis confirmed specificity. Standard curves were generated from ten-fold dilutions of DNA from *E. coli* to

calculate cell equivalents. Culturability was assessed in triplicate and viability in duplicate; statistical comparisons used Wilcoxon/Kruskal-Wallis and post-hoc Wilcoxon tests.

2.10 Gene expression study

The gene expression was studied using the prune-mimicking medium, selected based on the results of preliminary experiments.

2.10.1 Induction of osmotic stress

The CC and FI strains of *E. coli* were grown to mid-log phase, harvested, and resuspended in LB or prune-mimicking media. Cells were exposed to stress for 0, 0.5, 1, 3, and 16 hours. Cells were harvested by centrifugation, and stabilized in RNA Protect Solution and stored at 4 °C until later processing.

2.10.2 Gene expression analysis

The total RNA was extracted using the RNeasy Mini Kit, with TE buffer containing lysozyme and proteinase K to ensure efficient lysis and protein digestion. Purified RNA was eluted in RNase-free water and converted to complementary DNA (cDNA) using the QuantiTect Reverse Transcription Kit. Gene expression was quantified by SYBR Green-based qPCR in 25 µL reactions. The cycling program included an initial denaturation at 95°C for 10 minutes, followed by 40 cycles of denaturation at 95°C for 15 seconds, annealing at 57°C for 30 seconds, and extension at 72°C for 30 seconds. No template controls were included by substituting the template DNA with PCR-grade water. Fluorescence detection was performed at the end of each extension step. To verify product specificity, a melt curve analysis was performed following amplification. The relative gene expression was calculated using the ΔΔCt method, with *rpoB* as the reference housekeeping gene, and the fold change in gene expression was determined by the following equation: Fold change=$2^{-\Delta\Delta Ct}$.

3. RESULTS AND DISCUSSION

3.1 Determination of growth potential using turbidity-based methodology

E. coli growth experiments showed that both strains (CC, FI) grew well in LB and low-sugar media, while among the dried fruit–mimicking media, only the prune-mimicking medium supported growth. In this medium, OD values of both strains gradually increased over time, although the lag phase was extended, lasting approximately 32 hours for the FI strain and 85 hours for the CC strain when LB preadapted cells were transferred to prune-mimicking medium. In contrast, the cells that were pre-adapted in prune-mimicking medium and then transferred to LB medium exhibited much shorter lag phases (~3 hours for FI and ~6 hours for CC), highlighting the impact of both pre-adaptation and medium on growth dynamics. The pre-adaptation in LB or prune-mimicking medium influenced the growth dynamics. Even after prolonged pre-adaptation, bacterial growth occurred in prune-mimicking medium, indicating that this medium provides conditions suitable to *E. coli* persistence. These results align with previous studies showing that foodborne pathogens can survive on dried fruits for extended periods, particularly those offering more favourable moisture and sugar conditions.

3.2 Microbial diversity of dried fruits assessed via culture-dependent method

Using MALDI-TOF MS, 20 bacterial genera were identified across 30 dried fruit samples. The most frequently identified bacterial genera among all samples, in descending order of prevalence were *Bacillus*, *Priestia, Peribacillus, Micrococcus, Paenibacillus, Pantoea, Staphylococcus*, *Cytobacillus*, *Lysinibacillus* and *Niallia*. Ten genera were only found sporadically. Raisins exhibited the greatest bacterial diversity, followed by dried apricots, while prunes had the lowest microbial richness. This is likely due to their higher phenolic and antioxidant content, which inhibits bacterial growth. Many detected species, including *Bacillus*, *Paenibacillus*, *Curtobacterium*, *Lysinibacillus*, and *Metabacillus* are typical soil or plant-associated bacteria, indicating contamination during harvesting or handling. Several potential opportunistic pathogens were also found, such as *Staphylococcus epidermidis*, *Pantoea agglomerans*, *Pseudomonas* sp., *Enterococcus* sp., *Mixta calida*, and *Priestia flexa*, suggesting post-processing contamination and inadequate hygiene practices. While many of these bacteria have environmental or agricultural roles, their presence in ready-to-eat dried fruits is relevant for both spoilage and food safety. Fungal contamination was also prominent, as yeasts and moulds tolerate osmotic stress better than bacteria, however, Fungal identification was only possible in 27 % of dried apricot samples, 25 % of prune samples, however, it was possible in 72% of raisin samples. Fungal analysis of dried fruits revealed the frequent occurrence of osmotolerant yeasts, such as the *Zygosaccharomyces* species, across all fruit types. The highest fungal diversity was observed in raisins, from where a total of 11 fungal species were identified. Overall, dried fruits are susceptible to bacterial contamination due to open-air retail conditions and the lack of additional processing steps, such as washing or heat-treatment before consumption.

3.3 Additional tests for microbiota characterization

The pH of dried fruits varied across fruit types, with an average of 4.21 in dried apricots, 3.84 in prunes, and 3.83 in raisins. the pH values of dried apricot samples differed significantly from the pH values of prune ($Z = -5.55$, $p < 0.0001$, $\alpha = 0.05$) and raisin ($Z = -3.81$, $p = 0.0001$, $\alpha = 0.05$) samples. Within each fruit type, individual samples showed notable pH differences, which influenced microbial loads and diversity, though pH alone did not fully determine the microbiota composition. Samples with higher pH generally harboured more bacterial and fungal species, while samples with lower pH showed reduced microbial diversity.

Water activity (a_w) varied across the dried fruit types, with an average of 0.64 in dried apricots, 0.74 in prunes, and 0.45 in raisins. Certain samples with low-a_w still harboured multiple bacterial and fungal species, while certain samples with high-a_w showed lower microbial diversity, indicating that other factors, such as pH, handling, packaging, and antimicrobial compounds also affect microbial survival. These results align with previous studies showing that bacteria and pathogens can persist in foods with pH and low-a_w, but survival is modulated by multiple pre- and post-harvest factors.

Sugar concentrations varied across dried apricot, prune, and raisin samples and were generally lower than values found in the literature. This is likely due to the long-term storage prior to this experiment. Among the 30 dried fruit samples, seven samples were labelled as a product containing preservatives, in other cases, this data was not available. The preservative content was not analytically measured within this thesis, therefore the conclusions regarding the effect of the preservatives on the microbiota of these samples are made with this limitation. The microbiota of the preservative-treated samples differed considerably, however, these products did not always show reduced microbial diversity. Overall, the data indicate that the type of the preservative, the storage conditions, and the handling practice all influence microbial composition, with sulphur dioxide showing antimicrobial activity in some cases.

3.4 16S rDNA amplicon sequencing (culture-independent analysis)

Among the 30 commercially purchased dried fruit products, a total of seven bacterial genera were identified using the culture-independent method via 16S rDNA amplicon sequencing (*Bacillus*, *Priestia*, *Pantoea*, *Neobacillus*, *Tumebacillus*, *Cytobacillus*, *Mesobacillus*). *Bacillus* was also identified as the most predominant genus using the culture-independent approach. All the other genera detected via this method were also observed with the culture-dependent approach, with the exception of *Neobacillus*, *Mesobacillus*, *Tumebacillus*. Notably, *Neobacillus* and *Mesobacillus* were recently proposed as separate genera, reclassified from the genus *Bacillus*, as phylogenomic analyses revealed distinct monophyletic clades within this genus (Patel and Gupta; 2020). *Neobacillus* was identified from two dried apricot samples (Sample 7, 10). *Tumebacillus* was detected in one dried apricot (Sample 10) and in one raisin sample (Sample 23), while *Mesobacillus* was identified from a dried apricot sample (Sample 10). The samples were purchased

from different environments. Sample 7 was pre-packaged, while in contrast, Sample 10 was obtained from an open-air market, where fruits were stored in a partially open container, accessible only to the seller. Sample 23 also originates from a market where it was stored in an open container. These findings suggest that some members of these genera can withstand low-water activity and high temperature habitats, conditions similar to the desiccation process in dried fruits. As the number of newly identified species within these genera increases, the need for proper hygiene practices and post-harvest handling arises, especially given the potential emergence of pathogenic strains in the future.

3.5 Irradiation experiments

This section presents the irradiation experiments conducted to assess the effects of radiation on *E. coli* strains in different media.

3.5.1. Comparison of electron-beam (e-beam) and gamma irradiation

Two irradiation sources, electron beam and gamma rays, were compared to select the most suitable for further irradiation experiments. The D-value of *E. coli* was 0.2 kGy for e-beam irradiation, and 0.21 kGy for gamma irradiation. Since both treatments produced comparable inactivation efficiencies, e-beam irradiation was selected for later experiments. The results show that both irradiation types achieve a 1-log reduction under the tested conditions.

3.5.2 Determining the D-value of CC strain of E. coli with e-beam radiation

The e-beam irradiation of the CC strain of *E. coli* in LB medium yielded a D-value of 0.24 kGy. The increasing radiation doses led to the decline of viable cells, and at 2 kGy, the surviving population was reduced by approximately 8 logs. This indicates that e-beam irradiation is highly effective at inactivating *E. coli* under these conditions.

3.5.3 Assessing the behaviour of E. coli in osmotic environments upon gamma irradiation

Gamma irradiation experiments showed that the D-value of the CC strain of *E. coli* was elevated in media containing high concentrations of sugar (glucose, fructose, sucrose). Glucose offered the greatest protective effect, especially at 50 %, while fructose and sucrose provided moderate protection at higher concentrations. Low sugar levels (10 %) only had minimal impact on the D-value. These findings demonstrate that high sugar environments, such as found in dried fruits can enhance protection against irradiation. This highlights the need to study mixed-sugar food matrices for realistic risk assessment.

3.5.4 Determination of the D-values of CC and FI strains of E. coli using gamma irradiation

Both the CC and FI strains of *E. coli* exhibited identical D-values of 0.31 kGy in LB medium (control) when irradiated with gamma rays. In prune-mimicking medium, the D-values increased

slightly to 0.34 and 0.36 kGy, respectively, suggesting a modest protective effect against gamma radiation. Although modest, this protection reinforces that food matrix composition influences bacterial radiation tolerance.

3.6 Osmotic stress simulations

Osmotic stress simulations were performed to evaluate how high-sugar, low water activity environments that resemble the environments of different dried fruits (dried apricot, prune, raisin) influence the viability and culturability of *E. coli*. These experiments also assessed whether prior exposure to mild osmotic conditions alters the bacterial tolerance during subsequent osmotic stress.

3.6.1 Potential viable but non-culturable (VBNC)-like cells detected upon osmotic stress in dried fruit-mimicking media (viability and culturability assay)

Experiments using dried fruit-mimicking media showed that both *E. coli* strains (CC, FI) maintained their viability but lost their culturability, indicating a potential transition into a viable-but-non-culturable (VBNC)-like state under osmotic stress. While the control media (LB medium) supported stable growth, both *E. coli* strains exhibited declines in culturability within 24-96 hours in fruit-mimicking media. However, cells pre-adapted in fruit-mimicking media for extended periods were able to regain growth when transferred to nutrient-rich LB medium, suggesting that the apparent loss of culturability may reflect an extended lag phase or metabolic dormancy rather than a true VBNC state. These findings suggest that *E. coli* may become undetectable in dried fruits using conventional culture-based methods, but could be resuscitated in nutrient-rich foods, without additional processing steps, that would otherwise reduce microbial populations. An example of these foods is the overnight oats that are stored at room temperature rather than under refrigeration. Literature reports show similar behaviour in *Salmonella*, which also entered the VBNC state on dried fruits despite appearing non-recoverable by culture methods (Jayeola et al., 2022). The VBNC state of pathogens is an important challenge for food safety monitoring and risk assessment.

3.6.2 Osmotic stress simulations, investigating the effects of pre-adaptation in low-sugar environment

Pre-adaptation of *E. coli* to a low-sugar (0.35 M glucose) environment altered its subsequent response to dried fruit-mimicking media. The results showed an enhanced survival depending on the type of the media and the duration of stress exposure. Low-sugar adapted cells showed significantly higher cell counts in prune-mimicking medium after 4 hours ($Z = 2.5067$, $p = 0.0122$, $\alpha = 0.05$), and in raisin-mimicking medium after 24 hours ($Z = 2.0889$, $p = 0.0367$, $\alpha = 0.05$), while apricot-mimicking medium showed consistent but non-significant increases at both time points. These results indicate that even a brief exposure to moderate osmotic environments may improve the persistence of *E. coli* under otherwise inhibitory sugar concentrations. Given the widespread

consumption of dried fruits and the survival potential of *E. coli* in high-sugar matrices, osmotic pre-adaptation should be considered when evaluating microbial risks, as it may increase the likelihood of pathogen persistence in ready-to-eat food products.

3.7 Gene expression under osmotic stress

Both strains (CC, FI) of *E. coli* exhibited increased expression of *osmC* and *talA* within the first 30 minutes of exposure, followed by a decline at 1 hour. The expressions were then stabilized throughout the experiment. The third gene, *treA*, showed only a modest transient increase at 30 minutes. The observed expression levels were consistently lower in the FI strain compared to CC strain, suggesting that the food isolate is more adapted to hyperosmotic conditions. The observed spike in gene expression at 30 minutes might be due to the rapid activation of stress response mechanisms which stabilize cell homeostasis in *E. coli* upon transfer to sugar-enriched media, while the long-term adaptation mechanisms are initiated. As the cells adapt to the osmotic conditions, the expression of these genes declines. Alternatively, it is possible that the cells may not rely on these specific genes as prerequisite for their long-term survival.

4 CONCLUSIONS AND RECOMMENDATIONS

With the culture-dependent approach using MALDI-TOF MS for colony identification, 20 different genera were identified from 30 dried fruit samples. The three most frequently identified genera were *Bacillus* (from 21 samples), *Priestia* (from 12 samples), and *Peribacillus* (from 10 samples). Most of the isolates were environmental species such as *Bacillus amyloliquefaciens, B. licheniformis*, *B. pseudomycoides*, *B. mojavensis*, *B. thuringiensis*, *B. velezensis,* and *Curtobacterium flaccumfaciens*. However, potentially pathogenic bacteria, including *Staphylococcus epidermidis, Pantoea agglomerans, Enterococcus mundtii, Micrococcus luteus, Mixta calida* and *Priestia flexa* were also identified. Some members of the genera *Pantoea* and *Enterococcus*, on the other hand, can have beneficial roles in foods or natural environments, such as promoting plant growth, producing antimicrobial compounds, or contributing to fermentation processes, reflecting their dual potential to act as opportunistic pathogens or useful organisms. The culture-independent 16S rDNA amplicon sequencing identified *Bacillus* across 29 fruits samples, indicating broader detection coverage compared to culture-dependent approach. This method additionally revealed the presence of environmental contaminants (*Mesobacillus*, *Neobacillus* and *Tumebacillus*) not identified using MALDI-TOF MS, which could indicate the potential presence of VBNC bacteria in the dried fruit samples. Notably, *E. coli* was not detected with either method. In recent studies *E. coli* has been previously isolated from dried fruits, which demonstrates its ability to survive in low water activity environments, and has also been implicated in multiple outbreaks associated with low-moisture food products (Ntuli et al., 2017; Abed, 2025). Therefore, its detection would have allow the assessment of the microbiological safety of the samples, and the identification of potential post-processing contamination routes during handling and packaging. However, its presence in VBNC state or at levels below the detection limit cannot be excluded, as high abundance species could mask its presence, especially if its DNA was present in small amounts. In conclusion, the culture-dependent method revealed a more diverse microbiota, while the culture-independent provided complementary information. The results highlight that the comprehensive understanding of microbial diversity requires the combination of the culture-dependent and independent methods, as well as the combined interpretation of both richness and evenness metrics. The fungal presence was only assessed by the culture-dependent approach, which identified, by MALDI-TOF MS, two yeast species (*Zygosaccharomyces rouxii* and *Z. bailii*) and 13 species of moulds, belonging to 9 genera, including *Arthrinium*, *Aspergillus*, *Alternaria, Neoscytalidium, Scopulariopsis, Microsporum, Cladosporium, Penicillium,* and *Rhizopus.* The detection of pathogenic fungi (*Aspergillus niger* and *Arthrinium phaeospermum*) is concerning, as *A. niger* is known to produces mycotoxins, and both species have been associated with opportunistic human infections. However, *A. niger* is also widely used industrially for enzyme production. Notably, in dried fruits, fungal contamination is often of greater concern than bacterial contamination, as many fungi are capable of producing mycotoxins that can persist throughout processing and storage.

To investigate the behaviour of *E. coli* under osmotic stress, dried fruit-mimicking media (dried apricot, prune, raisin) with varying sugar compositions were prepared. The first goal was to determine whether *E. coli* can survive under these conditions. The growth experiment demonstrated that *E. coli* could grow in prune mimicking medium at 37 °C. The lag phase, however, was extended, lasting approximately 32 hours for the FI strain and 85 hours for the CC strain in the case where LB-pre-adapted cells were transferred to prune-mimicking medium. Moreover, an additional experiment was conducted which aimed to simulate the potential scenario of whether *E. coli* from unfavourable condition could regain its growth potential in favourable conditions. The cells that were pre-adapted in prune-mimicking medium and then transferred to the nutrient-rich LB medium exhibited much shorter lag phases (~3 hours for FI and ~6 hours for CC), and *E. coli* regained growth capacity. A similar scenario in everyday life would be the transfer of *E. coli* from dried fruits to yogurt or oats prepared with either milk or water.

The previously described model media were used to assess the induction of a potentially VBNC-like state of *E. coli* under osmotic stress. After 96 hours of exposure, culturability decreased significantly via the plate count method, whereas the viability, measured by PMA-qPCR, remained unchanged, indicating a VBNC-like behaviour. However, the actual viability was not verified, and no resuscitation step was performed, therefore the presence of VBNC cells cannot be confirmed, only inferred. Moreover, cells pre-adapted in prune-mimicking medium for 192 hours were able to regain their growth capacity upon transfer to LB medium, potentially indicating an extended lag phase, rather than true VBNC state.

The effect of pre-adaptation to osmotic environments was assessed in order to observe if the adaptation changes bacterial behaviour in media containing high sugar concentrations. It was observed that *E. coli* pre-exposed to moderate osmotic stress (0.35 M glucose) for 2.5 hours exhibited significantly higher cell counts in prune-mimicking medium after 4 hours of stress compared to the non-adapted cells ($Z = 2.5067$, $p = 0.0122$, $\alpha = 0.05$). However, after 24 hours, this difference diminished. In raisin mimicking medium, after 24 hours of stress, the pre-adapted cells had significantly higher cell counts compared to the non-adapted cells ($Z = 2.0889$, $p = 0.0367$, $\alpha = 0.05$). In apricot-mimicking medium, there was a considerable but not significant difference between the pre-adapted cells and the non-adapted cells at both 4 and 24 hours. This experiment revealed that the pre-adaptation gives a survival advantage to *E. coli* compared to non-adapted cells. Given the widespread consumption of dried fruits and the survival potential of *E. coli* in high-sugar matrices, osmotic pre-adaptation should be considered when evaluating microbial risks, as it may increase the likelihood of pathogen persistence in ready-to-eat food products.

Gene expression studies in the prune-mimicking medium demonstrated rapid transcriptional responses to osmotic stress. Within 30 minutes, the expression of all three tested genes (*osmC*,

talA, and *treA*) increased. The comparison of the strains from the culture collection and low water activity food revealed that the food isolate had greater tolerance to the high osmotic pressure. The observed spike in gene expression at 30 minutes might be due to the rapid activation of stress response mechanisms which stabilize cell homeostasis in *E. coli* upon transfer to sugar-enriched media, while the long-term adaptation mechanisms are initiated. As the cells adapt to the osmotic conditions, the expression of these genes declines. Alternatively, it is possible that the cells may not rely on these specific genes as prerequisite for their long-term survival.

The D-value of *E. coli* under gamma irradiation was assessed in media containing different concentrations of glucose, fructose or sucrose, as well as in the prune-mimicking medium. In prune-mimicking medium, both *E. coli* strains (CC, FI) were investigated. The results showed that high sugar concentrations increased the decimal radiation dose of *E. coli*, indicating that sugars might have protective effect. This has important implications for designing effective treatments for dried fruits and other high-sugar food products.

Based on the conclusions above, the following recommendations are proposed for future studies:

- Increase the sample size for microbiota characterisation,
- Optimize sample preparation protocols for both culture-dependent and culture-independent methods,
- Sequence the Internal Transcribed Spacer (ITS) region to enable fungal identification,
- Include PMA-qPCR analysis for improved culture-independent microbiota characterisation,
- Utilise 30 % glucose agar to facilitate the detection of osmotolerant microbiota,
- Investigate the matrix-effect in osmotic media, including pH adjustment and antioxidant content to better mimic dried fruit conditions,
- Conduct VBNC induction experiments under extended stress exposure (e.g., 2 weeks or longer), and include a resuscitation step and viability assays to confirm the presence of VBNC cells,
- Employ different pre-adaptation media to mimic other dried fruit products,
- Apply tighter sampling intervals to monitor early gene expressions (e.g., every 5 minutes during the first 30 minutes),
- Analyse broader stets of genes for transcriptomic analysis of *E. coli*,
- Investigate the effects of gamma irradiation on the gene expression of *E. coli* under osmotic stress
- Determine whether gamma irradiation induces the VBNC state in *E. coli*,
- Include multiple biological replicates in each experiment,
- Conduct experiments (e.g., growth measurement, stress experiments) at room temperature to more accurately reflect household storage conditions.

5 NEW SCIENTIFIC RESULTS

1. This is the first investigation on the microbiota of commercially available dried fruit products (dried apricot, prune, raisin) from Hungary and Austria using both culture-dependent (plating and MALDI-TOF MS) and culture-independent (16S rDNA amplicon sequencing) approaches.
   - Using the culture-dependent approach, several bacterial and fungal species were identified, including *Pantoea agglomerans*, *Enterococcus mundtii*, *Mixta calida*, *Priestia flexa*, *Aspergillus niger*, *Arthrinium phaeospermum* were identified from dried fruits samples, while the culture-independent approach revealed the presence of *Mesobacillus, Neobacillus,* and *Tumebacillus* genera. Some of the detected species, such as *Pantoea agglomerans*, *Enterococcus mundtii* and *Aspergillus niger* are known opportunistic pathogens, highlighting potential food safety concerns in dried fruit products.
   - Dried apricots and raisins exhibited higher bacterial diversity compared to prunes (on average 3.36 genera per sample in raisins, 2.70 genera per sample in dried apricots, and 1.62 genera per sample in prunes), likely due to the suppressive effects of phenolic compounds and antioxidants in prunes.
   - Raisins harboured the greatest number of unique bacterial genera (*Pseudomonas*, *Mixta*, *Curtobacterium*, *Kocuria*, *Niallia*, *Alkalihalobacillus*, *Ureibacillus* and *Metabacillus*), reflecting fruit-specific microbial niches.
   - The combined use of MALDI-TOF MS and 16S rDNA amplicon sequencing revealed the presence of both culturable and low-abundance taxa, demonstrating the importance of multi-approach profiling in low water activity food products (Dobó et al., 2025).
2. It was demonstrated for the first time that the pre-adaptation to a 0.35 M glucose containing medium influences the growth potential of *E. coli* strains.
   - Both a food isolate from a low-water activity product (buckwheat flour, FI strain) and a strain originated from culture collection (ATCC B.02031, CC strain), are able to grow in osmotic media containing 0.35 M glucose, and also in prune-mimicking medium (LB supplemented with 25.5 g/100 g glucose, 12.4 g/100 g fructose and 0.15 g/100 g sucrose). Furthermore, cells pre-adapted in prune-mimicking medium for 192 hours regained their growth capacity after transferring to LB medium, which provides a nutrient rich and low-osmotic environment, indicating resuscitation potential.
   - The pre-adaptation to 0.35 M glucose was shown to enhance the survival of *E. coli* (ATCC B.02031, CC strain) in dried fruit-mimicking media. The cell counts of the pre-adapted sample were significantly higher compared to the non-adapted controls in prune-mimicking medium after 4 hours of osmotic stress ($Z = 2.5067$, $p = 0.0122$, $\alpha = 0.05$) and in raisin-mimicking medium after 24 hours of osmotic stress ($Z = 2.0889$, $p = 0.0367$, $\alpha = 0.05$).

3. Exposure to diverse sugar concentrations resembling those found in dried apricot, prune and raisin may have induced a viable but non-culturable (VBNC)-like state in both the culture collection strain (ATCC B.02031, CC strain) and the low water activity food isolate (buckwheat flour, FI strain) of *E. coli*, over a 96-hour period. This presents the first evidence suggesting that conditions similar to those of dried fruits may trigger VBNC-like behaviour in *E. coli*. The potential VBNC-like state was inferred by comparing the results of the PMA-qPCR based viability assessments with the results of the conventional culturing (plate count) method. The significant difference between these two approaches observed between 24 and 96 hours in the dried fruit-mimicking media (CC strain of *E. coli* $Z = -2.95$, $p = 0.0031$; FI strain of *E. coli*: $Z = -2.16$, $p = 0.0304$; $\alpha = 0.05$) indicates the presence of metabolically active cells that were non-culturable under the tested conditions.
4. Gene expression studies revealed that the expression of three osmotic stress-related genes (*osmC*, *talA*, and *treA*) abruptly increased in the *E. coli* strains (culture collection: ATCC B.02031, CC; low water activity food isolate, from buckwheat flour, FI) after 30 minutes of osmotic stress in prune-mimicking medium. The food-originated (FI) strain presented greater tolerance to osmotic stress than the strain from culture collection (CC), presumably due to its origin from a low water activity environment.
5. It was demonstrated for the first time, that media with high osmotic pressure, containing high concentrations of glucose (30 %, 50 %), fructose (30 %, 50 %) or sucrose (50 %) showed a protective effect on the strain of *E. coli* from the culture collection (ATCC B.02031, CC) under gamma irradiation. The decimal radiation dose increased compared to the control medium:
   - in the case of glucose: from 0.32 kGy (control) to 0.41 kGy and 0.74 kGy (30 % and 50 % glucose, respectively),
   - in the case of fructose: from 0.33 kGy (control) to 0.41 kGy and 0.43 kGy (30 % and 50 %, respectively),
   - in the case of sucrose. from 0.32 kGy (control) to 0.42 kGy (50 %)

   The strongest protective effect was observed with the 50 % glucose. Similarly, the prune-mimicking medium with sugar concentrations and composition resembling the sugary environment of prunes also had a protective effect (moderate) on both the strain from the culture collection (ATCC B.02031) and the strain from the low water activity food isolate (buckwheat flour):

   - The decimal radiation doses were increased from 0.31 kGy (control) to 0.34 kGy for the culture collection strain, and to 0.36 kGy for the low water activity food isolate strain.

6 LIST OF PUBLICATIONS IN THE FIELD OF STUDY

Journal articles

First author publications

Dobó, V., Homlok, R., Mohácsi-Farkas, C., and Belák, Á. (2023). Effect of gamma irradiation, high sugar content and antimicrobials on survival of Escherichia coli: A review. Czech Journal of Food Sciences, 41(4), 231-247. https://doi.org/10.17221/235/2022-CJFS

Dobó, **V**., Wagner, E., Belák, Á., Peham, T., and Domig, K. J. (2025). Deciphering the microbial composition of dried fruits purchased from Austrian and Hungarian markets using culture-dependent and culture-independent methods. *Food Control*, 111845. https://doi.org/10.1016/j.foodcont.2025.111845

Conferences

Cefood 2024, Szeged, Hungary. **Dobó, V.,** Belák, Á., Mohácsi-Farkas, C. (poster: Microbiota of dried fruits and the osmotic stress of *E. coli*) 13-16 October 2024

The Miller Online Workshop on Radiation Chemistry. Homlok R., Kiskó G., Kovács A., Tóth T., **Dobó V**., Takács E., Mohácsi-Farkas Cs., Wojnárovits L., Szabó L. (poszter: Effect of electron beam irradiation and the presence of antibiotics on the population dynamics of resistant/sensitive bacterial cultures in model wastewater matrix). 10-12 February 2022

Third Research Coordination Meeting for CRP "Radiation Inactivation of Bio-hazards Using High Powered Electron Beam Accelerators" (International Atomic Energy Agency). Homlok R., Kiskó G., Kovács A., Tóth T., **Dobó V**., Takács E., Mohácsi-Farkas Cs., Wojnárovits L., Szabó L. (online. előadás: Effect of electron beam irradiation and the presence of antibiotics on the population ratio of resistant/sensitive bacterial in model wastewater matrix). 31 January - 04 February 2022

27th International Symposium on Analytical and Environmental Problems. Szeged, Magyarország. Homlok R., Kiskó G., Kovács A., Tóth T., **Dobó V**., Takács E., Mohácsi-Farkas Cs., Wojnárovits L., Szabó L. (poszter: Effect of electron beam irradiation and the presence of antibiotics on the population ratio of resistant/sensitive bacteria added prior to advanced oxidation treatment). 22-23 November 2021

A Magyar Tudomány Ünnepe. "Fiatal Talajbiológusok az élhető jövőért" Homlok R., Kiskó G., Kovács A., Tóth T., **Dobó V**., Takács E., Mohácsi-Farkas Cs., Wojnárovits L., Szabó L. (online előadás: Nagyhatékonyságú oxidációs kezelés és antibiotikumok hatása rezisztens/szenzitív baktériumkultúrák populációs arányára). 12 November 2021

Őszi Radiokémiai Napok, Balatonszárszó. Homlok R., Kiskó G., Kovács A., Tóth T., **Dobó V**., Takács E., Mohácsi-Farkas Cs., Wojnárovits L., Szabó L. (előadás: A környezeti szempontból jelentős koncentrációjú antibiotikumok hatása a rezisztens/szenzitív baktériumkultúrák populációs arányára, valamint kezelés gyorsított elektronokkal szennyvízmátrixban) 18-20 October 2021

MTA Élelmiszer-tudományi Bizottság 382. Tudományos kollokvium, Budapest. Homlok R., **Dobó V**., Kiskó G., Kovács A., Tóth T., Takács E., Mohácsi-Farkas Cs., Wojnárovits L., Szabó L. (előadás: Antibiotikum rezisztencia kialakulásának megelőzése szennyvízmátrixokban nagyenergiájú sugárzással). 28 May 2021

The Miller Online Workshop on Radiation Chemistry. Homlok R., Kiskó G., Kovács A., Tóth T., **Dobó V**., Takács E., Mohácsi-Farkas Cs., Wojnárovits L., Szabó L. (Online Flash prezentáció (3 perc előadás + 2 perc kérdés): Effect of electron beam irradiation and the presence of antibiotics on the population dynamics of resistant/sensitive bacterial cultures in model wastewater matrix with antibiotics and bacteria prior to advanced oxidation treatment). 10-12 February 2022

The Miller Online Workshop on Radiation Chemistry. Homlok R., Kiskó G., Kovács A., Tóth T., **Dobó V**., Takács E., Mohácsi-Farkas Cs., Wojnárovits L., Szabó L. (Flash video: Effect of electron beam irradiation and the presence of antibiotics on the population dynamics of resistant/sensitive bacterial cultures in model wastewater matrix with antibiotics and bacteria prior to advanced oxidation treatment). 10-12 February 2022

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